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REMARKS

By the present communication, claims 39-42 have been added to further define Applicants' invention with greater particularity. No new matter is introduced by the subject amendments as the new claim language is fully supported by the specification and original claims. For example, claim 39 is fully supported by the specification, page 22, line 25 to page 23, line 2, and page 30, lines 11-20. Therefore, claims 13-42 are currently pending in the instant application.

Applicants have recently recognized inaccuracies in the originally submitted sequence information. In light of this, a replacement Sequence Listing is submitted herewith, presenting the sequence information without the inaccuracies. The replacement sequence information provided herewith does not introduce new matter as all sequence information is supported by the attached copy of the Declaration of the inventors (as originally submitted in parent application U.S. Serial No. 09/227,718), verifying that they have been in continuous possession of the original clone of an exemplary invention receptor, and that the revised sequence information provided herewith is derived from the same clone from which the original sequence information was obtained. The replacement sequence listing corrects inaccuracies in the originally submitted sequence listing at four locations in SEQ ID NO:1 and two locations in SEQ ID NO:2, as follows: (i) at nucleotide position 1141, T instead of C, resulting in the codon for amino acid serine (in place of proline) at residue 187; (ii) at nucleotide positions 1224 and 1280, nucleotide corrections resulting in a reading frame shift in amino acids 215 to 233; (iii) at nucleotide 1872, T instead of C, resulting in no change in the encoded amino acid; and (iv) in the 3'-untranslated region, eleven single nucleotide corrections.

Applicants respectfully submit that all disclosed sequences with proper SEQ ID Nos. are included in the paper copy of the replacement Sequence Listing submitted herewith. A computer readable form (CRF) of the Sequence Listing and a Statement under 37 C.F.R. 1.821(f) and (g) will be submitted under separate cover.

Further, Applicants respectfully submit that the third paragraph on page 22 of the Specification has been amended to correct a typographical error. Support for the amendment is

found in the specification at page 11, lines 18-21, page 21, lines 10-12 and Figure 1A. Other amendments in the specification are made solely in accordance with 37 C.F.R. 1.821 – 1.825. No new matter is introduced by the amendments. Therefore, entry of the amendments is respectfully requested.

CONCLUSION

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. In the event any matters remain to be resolved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

Date 1/24/03

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Enclosures: APPENDICES A and B
Declaration by Inventors
Sequence Listing

APPENDIX A: Marked-up Version of the Specification to Show Changes Made

Paragraph on page 13, lines 16-23:

-- **Figure 6A** presents a schematic comparison of nucleotide sequences encoding response elements found in inducible cytochrome P450 enzymes **(SEQ ID NOS 3-11, respectively in order of appearance)**. A database search for repeats of the sequence RGKTCA **(SEQ ID NO: 41)** was performed and some of the matches for enzymes involved in hepatic steroid hydroxylation are indicated. The standard nomenclature for P450 enzymes has been utilized. P450R is the single P450 oxidoreductase required for hydroxylation of steroids. UGT1A6 is a rat uridine diphosphate (UDP)-glucuronosyltransferase that conjugates glucuronic acid to hydroxylated steroids. –

Paragraph on page 13, line 24 through page 14, line 2:

-- **Figure 6B** presents a schematic comparison of conserved glucocorticoid-response elements found in human CYP3 genes. The region of human CYP3A4 **(SEQ ID NO: 33)** shown is necessary and sufficient for glucocorticoid and rifampicin induction of the full-length promoter. Corresponding regions of CYP3A5 **(SEQ ID NO: 34)** and CYP3A7 **(SEQ ID NO: 35)** are shown (Barwick et al., *Mol. Pharmacol.* 50:10-16, 1996).
--

Paragraph on page 15, lines 18-26:

-- Figure 8C illustrates that the DR-3 element is essential for SXR-mediated activation of CYP3A2, and is interchangeable with the IR-6 element. The wild type (DR3/WT **(SEQ ID NO:39)**, filled bars) or mutant forms (DR3/M1 **(SEQ ID NO:42)**, open bars; DR3/M2 **(SEQ ID NO:43)**, stippled bars; and DR3/IR6, hatched bars) of CYP3A23 cellular promoter reporters were transfected into primary rat hepatocytes in the presence of expression vector for SXR. The ligand treatment and data presentation are the same as in 8A. RIF, rifampicin; CTZ, clotrimazole. Note the disruptions of DR-3 element

(DR3/M1, and DR3/M2) abrogate the activation of CYP3A23, and the replacement of DR-3 element with IR-6 element (DR3/IR3) rescue the responsiveness. --

Paragraph on page 22, lines 10-15:

-- Thus, the terms "SXR receptor" and "SXR polypeptide" are interchangeable as used herein and are intended to include functional fragments of the invention SXR polypeptide(s). Such fragments include peptides having the DNA binding and/or the ligand binding properties of SXR, e.g. the DNA binding domain thereof (e.g. amino acid residues ~~[71]~~ 41-107 as shown in SEQ ID NO:2), the ligand binding domain thereof (e.g., amino acid residues 141-434 as shown in SEQ ID NO:2). --

Paragraph on page 23, lines 11-20:

-- Examples of response elements suitable for use in practice of the invention methods can be selected from the following:

DR-3,4,5=AGGTCAN$_n$AGGTCA, wherein n is 3 **(SEQ ID NO: 44)**, 4 **(SEQ ID NO: 45)**, or 5 **(SEQ ID NO: 46)** ~~**[(SEQ ID NOS: 15, 16 and 17)]**~~;

βDR-3,4,5=AGTTCAN$_n$TGAACT, wherein n is 3 **(SEQ ID NO: 22)**, 4 **(SEQ ID NO: 47)** or 5 **(SEQ ID NO: 48)** ~~**[(SEQ ID NO:22)]**~~ and

IR-6 = TGAACTN$_n$AGGTCA, wherein n is 6 (SEQ ID NO: 23), and the like.

Those of skill in the art will recognize that any combination of nucleotides can be used to make up the 3, 4, 5, or 6 nucleotide spacer between the repeated half sites (i.e., N$_n$ in SEQ ID NOS: ~~[15, 16, 17]~~ 44, 45, 46, 22, 47, 48 or 23). --

Paragraph on page 57, lines 24-27:

-- CYP3A oligonucleotides tested had the following sequences:

CYP3A4, tagaataTGAACTcaaaggAGGTCAgtgagtgg (SEQ ID NO: ~~[31]~~ 33);

CYP3A5, tagaataTGAACTcaaaggAGGTAAgcaaaggg (SEQ ID NO: ~~[32]~~ 34); and

CYP3A7, tagaataTTAACTcaatggAGGCAgtgagtgg (SEQ ID NO: ~~[33]~~ 35). --

Paragraph on page 60, lines 3-17:

-- The fact that SXR is necessary and sufficient to render the induction of both human CYP3A4 and rat CYP3A23 gene in rodent hepatocytes by RIF suggested that the host cellular environment, SXR/PXR herein, rather than the gene structure, dictates the patterns of inducibility of CYP3A genes. The above notion would predict: (1) The SXR/PXR response element is essential for the activation of CYP3A genes; and (2) The response elements of SXR and PXR are interchangeable. Therefore, mutagenesis analysis was performed on the promoter of the rat CYP3A23 gene to examine these predictions. In vitro electrophoretic mobility shift assays showed that both SXR:RXR and PXR:RXR heterodimers efficiently bind to the DR-3 elements (5'TGAACTtcaTGAACT 3' (SEQ ID NO: 39)) in the CYP3A23 promoter (Blumberg et al., 1998). As shown in Figure 8C, mutation of both half sites (DR3/M1) or a single half site (DR3/M2) abolished the PXR and/or SXR-mediated activation by PCN, RIF, and CTZ; on the other hand, replacement of the wild type DR-3 element by an IR-6 element of the human CYP3A4 gene promoter (Blumberg et al., 1998, and Kliewer et al., 1998) successfully rescue the inducibility by PCN, RIF and CTZ. --

Paragraph on page 61, lines 8-20:

-- Genomic DNA was isolated as described before (Xie et al., 1999). The polymerase chain reaction (PCR) was used to screen the transgene positive mice. Two oligonucleotides used to screen Alb-SXR mice are 5'-GAGCAATTCGCATTACTCTGAAGT-3' (SEQ ID NO: 36) (annealing to SXR cDNA), and 5'-GTCCTTGGGGTCTTCTACCTTTCTC-3' (SEQ ID NO: 37) (annealing to the SV 40 sequence downstream of the transgene in the transgene cassette). Another two oligonucleotides used to screen Alb-VPSXR are 5'-GACGATTTGGATCTGGACATGTTGG-3' (SEQ ID NO: 38) (annealing to VP16 sequence), and 5'-GTTTTCATCTGAGCGTCCATCAGCT-3' (SEQ ID NO: 40)

(annealing to the SXR cDNA). PCR was carried out in a DNA thermal cycler (Perkin-Elmer/Cetus) using the following program: 94°C for 1 min, 58°C for 2 min, and 72°C for 3 min and products were analyzed by electrophoresis on a 1% agarose gel. The transgene integration status was analyzed by Southern blot using transgene specific probes as described before (Xie et al., 1999). --

APPENDIX B: **Claims as They Will Stand Upon Entry of the Amendment**

13. (Reiterated) A transgenic mouse whose genome contains a transgene comprising a gene encoding a human steroid and xenobiotic receptor (SXR) polypeptide operably linked to an inducible promoter/enhancer,

wherein said SXR polypeptide is a member of the steroid/thyroid hormone superfamily and forms a heterodimer with retinoid X receptor,

wherein said SXR polypeptide binds to a direct or inverted repeat response element based on the half site RGBNNM,

wherein:

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence AGTTCA;

wherein said SXR polypeptide inducibly activates transcription in response to a wide variety of natural and synthetic steroid hormones, including at least compounds that induce catabolic enzymes, steroid receptor agonists and antagonists, and bioactive dietary compounds, and

wherein said transgenic mouse expresses said SXR polypeptide in at least one of the liver and intestine.

14. (Reiterated) A transgenic mouse according to claim 13, wherein expression of said SXR polypeptide in at least one of the liver and intestine activates in the transgenic mouse a response to natural and synthetic steroid hormones to which a wild type mouse does not respond.

15. (Reiterated) A transgenic mouse according to claim 13, wherein said SXR polypeptide comprises an SXR ligand binding domain and a DNA binding domain obtained from a transcription activating factor.

16. (Reiterated) A transgenic mouse according to claim 13, wherein the ligand binding domain and DNA binding domain of said SXR polypeptide are obtained from SXR.

17. (Reiterated) A transgenic mouse according to claim 13, wherein said mouse is further transformed with a vector which comprises:

(a) a promoter that is operable in said mouse,

(b) a hormone response element, and

(c) DNA encoding a protein,

wherein said protein-encoding DNA is operatively linked to said promoter for transcription of said DNA, and

wherein said promoter is operatively linked to said hormone response element for activation thereof.

18. (Reiterated) A transgenic mouse according to claim 17, wherein said protein is a reporter.

19. (Reiterated) A transgenic mouse according to claim 17, wherein said protein is a mammalian cytochrome p450.

20. (Reiterated) A transgenic mouse according to claim 13, wherein the promoter/enhancer is the albumin promoter/enhancer.

21. (Reiterated) A transgenic mouse according to claim 13, wherein the transgene further comprises nucleic acid sequence encoding VP16.

22. (Reiterated) Cells derived from a transgenic mouse according to claim 13.

23. (Reiterated) A transgenic knock-out mouse whose genome comprises a homozygous disruption in an endogenous SXR polypeptide gene, wherein said homozygous disruption prevents function of an endogenous SXR polypeptide and results in said transgenic knockout mouse exhibiting decreased response to steroids and xenobiotics as compared to a wild-type mouse.

24. (Reiterated) A transgenic mouse whose genome contains a transgene comprising a gene encoding a human steroid and xenobiotic receptor (SXR) polypeptide operably linked to a constitutively active promoter/enhancer,

wherein said SXR polypeptide is a member of the steroid/thyroid hormone superfamily and forms a heterodimer with retinoid X receptor,

wherein said SXR polypeptide binds to a direct or inverted repeat response element based on the half site RGBNNM,

wherein:

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence AGTTCA;

wherein SXR polypeptide inducibly activates transcription in response to a wide variety of natural and synthetic steroid hormones, including at least compounds that induce catabolic enzymes, steroid receptor agonists and antagonists, and bioactive dietary compounds, and

wherein said transgenic mouse expresses said SXR polypeptide in at least one of the liver and intestine.

25. (Reiterated) A transgenic mouse according to claim 24, wherein constitutive expression of said SXR polypeptide results in growth retardation and hepatomegaly in said mouse.

26. (Reiterated) A transgenic mouse according to claim 24, wherein said SXR polypeptide comprises an SXR ligand binding domain and a DNA binding domain obtained from a transcription activating factor.

27. (Reiterated) A transgenic mouse according to claim 24, wherein the ligand binding domain and DNA binding domain of said SXR polypeptide are obtained from SXR.

28. (Reiterated) A transgenic mouse according to claim 24, wherein said mouse is further transformed with a vector which comprises:

(a) a promoter that is operable in said mouse,

(b) a hormone response element, and

(c) DNA encoding a protein,

wherein said protein-encoding DNA is operatively linked to said promoter for transcription of said DNA, and

wherein said promoter is operatively linked to said hormone response element for activation thereof.

29. (Reiterated) A transgenic mouse according to claim 28, wherein said protein is a reporter.

30. (Reiterated) A transgenic mouse according to claim 28, wherein said protein is a mammalian cytochrome p450.

31. (Reiterated) A transgenic mouse according to claim 28, wherein the response element in the reporter vector is based on the half site RGBNNM,

wherein:

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence AGTTCA.

32. (Reiterated) A transgenic mouse according to claim 24, wherein the promoter/enhancer is the VP16 promoter/enhancer.

33. (Reiterated) A transgenic mouse according to claim 24, wherein the transgene further comprises nucleic acid sequence encoding VP16.

34. (Reiterated) Cells derived from a transgenic mouse according to claim 24.

35. (Reiterated) A method for producing a transgenic mouse, said method comprising:

injecting a one-cell mouse zygote with a transgene comprising a gene encoding a human steroid and xenobiotic receptor (SXR) polypeptide operably linked to an inducible or a constitutively active promoter/enhancer,

wherein said SXR polypeptide is a member of the steroid/thyroid hormone superfamily and forms a heterodimer with retinoid X receptor,

wherein said SXR polypeptide binds to a direct or inverted repeat response element based on the half site RGBNNM,

wherein:

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence AGTTCA, and inducibly or constitutively activates transcription in response to a wide variety of natural and synthetic steroid hormones, including at least compounds that induce catabolic enzymes, steroid receptor agonists and antagonists, and bioactive dietary compounds, and

wherein said polypeptide is detectably expressed in at least one of the liver and the intestine, and ,

obtaining from the zygote a transgenic mouse that expresses said SXR polypeptide in the liver.

36. (Reiterated) A method according to claim 35, wherein expression of said SXR polypeptide activates in the transgenic mouse a response to natural and synthetic steroid hormones to which a wild type mouse does not respond.

37. (Reiterated) A method according to claim 35, wherein the promoter/enhancer is an inducible promoter/enhancer.

38. (Reiterated) A method according to claim 35, wherein the promoter/enhancer is a constitutively active promoter/enhancer.

39. (New) A transgenic mouse whose genome contains a transgene comprising a gene encoding a human steroid xenobiotic receptor (SXR) polypeptide, or functional fragment of said polypeptide, operably linked to an inducible tissue-specific promoter/enhancer,

wherein said human SXR polypeptide is inducibly expressed in said transgenic mouse in a tissue-specific manner,

wherein said human SXR polypeptide is a member of the steroid/thyroid hormone superfamily and forms a heterodimer with retinoid X receptor,

wherein said heterodimer binds to a direct or inverted repeat response element comprising at least two half sites RGBNNM separated by a spacer of 0 up to 15 nucleotides,

wherein:

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said RGBNNM sequence are identical with the nucleotides at corresponding positions of the sequence AGTTCA; and

wherein compounds selected from the group consisting of natural and synthetic steroid hormones including at least compounds that induce catabolic enzymes, steroid receptor agonists and antagonists, and bioactive dietary compounds, interact with said human SXR polypeptide directly or indirectly to activate transcription of a gene under the control of a cytochrome P450 response element therefore.

40. (New) A transgenic knock-out mouse whose genome comprises a homozygous disruption in an endogenous mouse SXR polypeptide gene, wherein said homozygous disruption comprises insertion, deletion or point mutation of said mouse SXR polypeptide, wherein said disruption results in a decrease in transcription of a gene under the control of a cytochrome P450 response element mediated by said mouse SXR polypeptide in said transgenic knockout mouse as compared to a wild-type mouse.

41. (New) A method for producing a transgenic mouse, said method comprising:

injecting a one-cell mouse zygote with a transgene comprising a gene encoding a human steroid xenobiotic receptor (SXR) polypeptide, or functional fragment of said polypeptide, operably linked to an inducible or a constitutively active tissue-specific promoter/enhancer,

wherein said human SXR polypeptide is inducibly or constitutively expressed in said transgenic mouse in a tissue-specific manner,

wherein said human SXR polypeptide is a member of steroid/thyroid hormone superfamily and forms a heterodimer with retinoid X receptor,

wherein said heterodimer binds to a direct or inverted repeat response element comprising at least two half sites RGBNNM separated by a spacer of 0 up to 15 nucleotides,

wherein:

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said - RGBNNM - sequence are identical with nucleotides at corresponding positions of the sequence AGTTCA,

wherein compounds selected from the group consisting of natural and synthetic steroid hormones including at least compounds that induce catabolic enzymes, steroid receptor agonists and antagonists, and bioactive dietary compounds, interact with said human SXR polypeptide directly or indirectly to activate transcription of a gene under the control of a cytochrome P450 response element therefore, and

obtaining a transgenic mouse from said mouse zygote, wherein said transgene is incorporated into the genome of said transgenic mouse and wherein said transgenic mouse expresses said human SXR polypeptide.

42. (New) A transgenic mouse whose genome contains a transgene comprising a gene encoding a human steroid xenobiotic receptor (SXR) polypeptide, or functional fragments of said polypeptide, operably linked to a constitutively active tissue-specific promoter/enhancer,

wherein said human SXR polypeptide is constitutively expressed in said transgenic mouse in a tissue-specific manner,

wherein said human SXR polypeptide is a member of the steroid/thyroid hormone superfamily and forms a heterodimer with retinoid X receptor,

wherein said heterodimer binds to a direct or inverted repeat response element comprising at least two half sites RGBNNM separated by a spacer of 0 up to 15 nucleotides,

wherein:

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said RGBNNM sequence are identical with the nucleotides at corresponding positions of the sequence AGTTCA; and

wherein compounds selected from the group consisting of natural and synthetic steroid hormones including at least compounds that induce catabolic enzymes, steroid receptor agonists and antagonists, and bioactive dietary compounds, interact with said human SXR polypeptide directly or indirectly to activate transcription of a gene under the control of a cytochrome P450 response element therefore.